

Conversion of corn milling fibrous co-products into ethanol by recombinant *Escherichia coli* strains K011 and SL40

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Corn hulls and corn germ meal were both evaluated as feedstocks for production of ethanol for biofuel. Currently, these fibrous co-products are combined with corn steep liquor and the fermentation bottoms (if available) and marketed as cattle feed. Samples were obtained from wet and dry corn mills. The corn hulls and germ meal were evaluated for starch and hemicellulose compositions. Starch contents were 12 to 32% w/w and hemicellulose (arabinoxylans) contents were 23 to 64% w/w. Corn fibrous samples were hydrolysed, using dilute sulphuric acid, into mixed sugar streams containing arabinose, glucose and xylose. Total sugar concentrations in the hydrolysate varied from 8.4 to 10.8% w/v. The hydrolysates were fermented to ethanol using recombinant *E. coli* strains K011 and SL40. Ethanol yields were 0.38 to 0.41 g ethanol produced/g total sugars consumed and fermentations were completed in 60 h or less. However, residual xylose was detected for each hydrolysate fermentation and was especially significant for fermentations using strain SL40. Strain K011 was a superior ethanologenic strain compared with strain SL40 in terms of both ethanol yield and maximum productivity.

Key words: Alcohol, biofuel, corn fibre, corn germ, *Escherichia coli*, fermentation.

In 1994, about 1.3 billion gallons of fuel ethanol were produced in the USA (House 1995), of which 95% was derived from fermentation of corn starch. Opportunity exists for further increasing ethanol's share of the automotive fuel market because of increased attention to clean air and oxygenates for fuels and decreasing domestic petrochemical reserves. However, increasing ethanol production will require using alternative feedstocks. Only lignocellulosic biomasses are plentiful and inexpensive enough to be possible competitors with starch as a fermentation feedstock (Hacking 1986). Particularly promising lignocellulosic fermentation feedstocks are fi-

brous residues generated from corn milling because they are high in carbohydrates, stockpiled in central locations (including in some cases at ethanol fermentation facilities), fairly homogeneous in composition, and low in naturally occurring microbial inhibitors.

Corn fibre residues are generated from both dry and wet corn milling operations. The primary sources of these fibres are corn hulls or bran and de-oiled germs (Gulati *et al.* 1996). The former is termed corn germ cake when the oil has been expelled and corn germ meal when the oil has been extracted (Wright 1987). Corn bran generated by wet milling is called corn fibre. The corn milling industry generated in excess of 2.4 million metric tons (dry basis) of corn bran/fibre and 0.94 million metric tons (dry basis) of corn germ cake/meal in 1994 (Gulati *et al.* 1996).

Conversion of corn hulls and germ meal into ethanol requires two steps: pretreatment and fermentation. In the pretreatment step, the carbohydrate polymers are converted into free sugars. The starch and hemicellulose components of corn fibre residues can be easily and efficiently hydrolysed using a combination of weak acid

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* Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

and high temperature (Dunning & Lathrop 1945; McMillan 1994). The free sugars produced are a mixture of pentoses (arabinose and xylose) and hexoses (galactose and glucose). Fermentation of these hydrolysates is problematic, however, because *Saccharomyces cerevisiae* and *Zymomonas mobilis* (microorganisms currently used for industrial ethanol fermentations) do not ferment pentoses. In fact, only two wild-type microorganisms (*Sarcina ventriculi* and *Paecilomyces* sp. NF1) have been discovered that efficiently ferment both arabinose and xylose into ethanol, but each has shortcomings that preclude them from industrial use (Bringer *et al.* 1984; Finn *et al.* 1984; Wu *et al.* 1986; McMillan & Boynton 1994; Dien *et al.* 1996). However, in the last decade, a variety of recombinant microorganisms have been developed by Ingram and his colleagues that efficiently ferment hexose and pentose sugar mixtures (Ingram *et al.* 1987; Altermann & Ingram 1989; Ohta *et al.* 1991; Lindsay *et al.* 1995). For this study, we have used two of these, *E. coli* strains K011 and SL40. *E. coli* strain K011 contains genes from the ethanol fermentation pathway of *Z. mobilis* (pyruvate decarboxylase and alcohol dehydrogenase) integrated into its chromosome (Ohta *et al.* 1991). Strain K011 has also been genetically modified to eliminate succinate production, a competing fermentation pathway (Ohta *et al.* 1991). The other *E. coli* strain, SL40, is a fosfomycin-resistant mutant of strain K011 that has been previously reported to have increased ethanol yield for high initial xylose concentrations (12% w/v) and increased productivity compared to its parent (Lindsay *et al.* 1995).

Three fibrous co-products were tested as potential feedstocks: corn fibre, dried milled corn bran (DietFiber®), and germ meal. Each was converted into concentrated sugar solutions (8% w/v or greater) using weak acid hydrolysis and fermented (separately) with strains K011 and SL40. Fermentation results were compared for ethanol production, productivity, yield, and sugar utilization. This is the first time, to our knowledge, that coarse DietFiber® and germ meal hydrolysates have been tested as fermentation feedstocks.

Materials and Methods

Sources of Corn Co-Products and Chemicals

The DietFiber® and corn germ meal were obtained from a corn dry milling plant (Lauhoff Grain Co, Danville, IL), and the corn fibre was obtained from a corn wet milling plant (Pekin Energy, Pekin, IL). Sugars used for xylose and mixed sugar fermentations were supplied by Sigma (St. Louis, MO), other media components by Difco Laboratories (Detroit, MI) and chemicals by Fisher Scientific (Fair Lawn, NJ).

Compositional Analysis of Corn Biomasses

Approximately 5 mg samples of each corn co-product was treated with 2 ml of 2 M trifluoroacetic anhydride (TFA) at

100 °C for 1 h. The TFA was evaporated at 50 °C with a stream of N₂. The residue was resuspended in 0.5 ml of distilled H₂O and the water evaporated. Samples were resuspended in 1 ml of distilled H₂O and analysed for sugars. Sugar standards for glucose, xylose, and arabinose were treated according to the same protocol, in order to correct for sugar decomposition.

Dilute Acid Hydrolysis of Corn Residues

The corn fibre was dried at 65 °C for approximately 24 h and ground in a hammer mill until it passed through a 28 mesh sized screen. The coarse dietary fibre and corn germ meal were used as received; their moisture contents were 7.1% and 9.6%. The residues were mixed with 1% v/v H₂SO₄ at a ratio of 1 g biomass to 4.25 ml, placed in a shallow Pyrex dish, covered with aluminum foil, and heated to and maintained at 121 °C for either 1 h (corn fibre and corn germ meal) or 30 min (DietFiber®). These cooking times had been optimized for maximum free sugar recovery in preliminary experiments (data not shown). After hydrolysis, insoluble material was removed by filtration, the remaining hydrolysate neutralized to pH 6.5 by adding Ca(OH)₂, and the resulting gypsum (Ca(SO₄)) removed by filtration. The following medium components were added to the recovered hydrolysate (g/l): yeast extract, 5; tryptone, 10; NaCl, 5. Antifoam A [1.0 ml of a 10% (w/v) solution/l (Sigma Chemical Co.)] and 40 mg chloramphenicol/l (Cm) were also added. The medium was split into 200 ml aliquots and inoculated immediately with either strain K011 or SL40.

Bacterial Strains

E. coli strains K011 and SL40 were supplied by Dr L.O. Ingram. They were grown on amended LB broth [(g/l): yeast extract, 5; tryptone, 10; NaCl, 5] supplemented with 40 mg Cm/l and either 50 g xylose/l for liquid cultures or 20 g xylose and 15 g agar/l for solid medium. Sugars were filter-sterilized and the other ingredients were autoclaved. The strains were stored in glycerol stocks (50% v/v) at -80 °C after overnight growth on amended LB.

Preparation of Inoculum

Each fermentation was started by plating the two *E. coli* strains from frozen cultures onto LB plates supplemented with xylose and Cm. A large single colony was chosen from each plate and used to inoculate 50 ml LB supplemented with xylose and Cm. The culture was incubated for approximately 24 h at 30 °C with agitation at 90 rev/min on a rotary shaker. Cells were harvested by centrifugation (12,000 × g, 10 min) and resuspended in 4 ml sterile basal LB medium. Each fermentation culture was inoculated with 0.15 mg cells/ml of medium; an optical density of 1.0 at 550 nm was determined to be equal to a concentration of 0.30 mg cell dry weight/ml.

Fermentation Experiment

Fermentations were carried out in automatic pH-controlled 500-ml Fleakers® (Cole Parmer) with a working volume of 200 ml as previously described (Beall *et al.* 1991). Fermentations were maintained at pH 6.5 by the addition of KOH (2M) and were carried out at 35 °C. Cultures were stirred magnetically, using 1.5 inch stir-bars, at 200 rev/min. Samples were withdrawn periodically to measure optical density, ethanol, sugar and organic acid concentrations.

Analytical Methods

Ethanol concentrations were measured by gas-liquid chromatography using a Hewlett Packard 5890A gas chromatograph

(Hewlett Packard Co, Wilmington, DL) equipped with an 80/100 Porapak Q column (6 ft \times 1.8 in, Supelco, Bellefonte, PA) and an FID detector. The injection, oven and detector temperatures were set at 200, 165 and 250 °C respectively.

Sugars (arabinose, glucose, galactose, and xylose) were determined by HPLC with an HPX-87C column (300 \times 7.8 mm; Bio-Rad Laboratories, Hercules, CA) at 85 °C. The column was eluted with distilled water at a flow rate of 0.6 ml/min. The detector used was a 410 Differential Refractometer (Milford, MA). Xylose and galactose co-elute on this column, precluding determination of their individual sugar concentrations. However, galactose is a minor component of corn fibre and corn germ hemicellulose (Hespell *et al.* 1996). Organic acids (lactate, acetate, and succinate) were measured also by HPLC, but using a HPX-87H column (300 \times 7.8 mm; Bio-Rad Laboratories, Hercules, CA) at 65 °C and eluted with 20 mM sulphuric acid (0.6 ml/min).

Calculation of Fermentation Parameters

Ethanol yields were calculated by dividing the amount of ethanol produced by the total amount of sugar consumed. Maximum volumetric ethanol production rates (V_{ETOH}) were calculated from the increase in ethanol concentration between subsequent samples. Both the yield and maximum V_{ETOH} were corrected for the dilution of the culture by the automatic base additions needed to maintain pH 6.5 and sampling volume withdrawn (Beall *et al.* 1991).

A carbon balance was carried out based upon consumed sugars (arabinose, glucose, and xylose + galactose) and products formed (acetate, biomass, ethanol, lactate, and succinate). It was assumed that the amount of carbon dioxide produced was equimolar to the ethanol and acetate minus the succinate production (Clark 1989). The dried *E. coli* biomass was assumed to be 45% w/w carbon (Bauer & Ziv 1976). Final product and sugar concentrations were corrected for dilution by base addition.

Results

Composition of Corn Milling Co-products

The corn fibre, coarse DietFiber®, and corn germ meal were analysed for neutral sugar composition, excluding cellulose (Table 1). Each contained over 50% non-cell-

ulosic carbohydrates, but composition varied widely. Corn germ meal and corn fibre contained large amounts of starch; 0.32 g and 0.22 g starch/g dry weight, respectively. The DietFiber® contained much less starch (0.12 g/g), but was richest in total non-cellulosic carbohydrates (76.6% w/w). The ratio of xylose + galactose to arabinose for the DietFiber® and corn fibre were almost equal (1.4 and 1.5 respectively), which was to be expected since the source of hemicellulose in both of these is the corn hulls. In contrast, the xylose + galactose to arabinose ratio for the corn germ meal was 0.90, signifying that this hemicellulose is relatively richer in arabinose than hemicellulose from corn hulls.

Weak Acid Hydrolysis of Corn Milling Co-Products

The feedstocks were hydrolysed with 1% (v/v) H_2SO_4 at 121 °C. Optimal digestion times were determined to be 1 h for corn germ meal or corn fibre and 30 min for DietFiber® (data not shown). Following hydrolysis, insolubles were removed by filtration and hydrolysates neutralized to pH 6.5 with $\text{Ca}(\text{OH})_2$. Insoluble CaSO_4 was removed by filtration before fermentation.

All hydrolysates contained a mixture of sugars including: arabinose, glucose, and xylose + galactose (Table 2). Total sugar concentrations varied with feedstock and were 8.4% to 10.8% w/v. The DietFiber® and corn fibre hydrolysates were rich in xylose + galactose, but still had appreciable amounts of arabinose and glucose. The corn germ meal contained primarily glucose and equal amounts of arabinose and xylose + galactose. Relative amounts of individual sugars recovered in each hydrolysate were similar to those determined before acid-treatment. In addition, the DietFiber® hydrolysate contained 4.9 g acetate/l and the corn fibre contained 3.7 g acetate/l. No acetate was detected in the corn germ meal hydrolysate.

Table 1. Composition of wet and dry corn milling co-products.

Composition	Total neutral sugars (%) [*]	Glucose	Xylose [†]	Arabinose
Coarse DietFiber®	76.6 \pm 5.3	16.0 \pm 2.2 [‡]	49.6 \pm 2.8 [‡]	34.3 \pm 2.4 [‡]
Corn germ meal	55.2 \pm 4.0	58.0 \pm 8.3	19.9 \pm 5.8	22.1 \pm 5.2
Corn fibre	68.3 \pm 1.1	31.5 \pm 1.9	41.0 \pm 0.9	27.5 \pm 1.3

^{*} Percent total neutral sugars represents g sugars/g dry biomass (excludes glucose derived from cellulose).

[†] Values for glucose, xylose and arabinose are expressed as a percentage of the total neutral sugars.

[‡] Values for xylose include galactose, which constitutes approximately 5% of the total neutral sugars. Values represent the means \pm standard deviation.

Table 2. Compositions of hydrolysates and sugars used for fermentations.

Substrate	Glucose	Xylose [†]	Arabinose	Total [*] Sugar	Recovery [‡] (%)
Coarse DietFiber®	2.17	5.99	2.65	10.82	69.5
Corn germ meal	5.33	1.39	1.70	8.41	77.5
Corn fibre	2.61	4.09	2.28	8.98	66.1
Mixed sugars	4.00	4.00	2.00	10.00	NA
Xylose	0.00	10.00	0.00	10.00	NA

^{*} Values for glucose, xylose, arabinose and total sugar are expressed as a percentage of the total neutral sugars.

[†] Values for xylose also include galactose, which constitutes approximately 5% of the total neutral sugars.

[‡] Percent recovery represents g sugars recovered/g sugars present in biomass (excluding cellulose). Losses exclude those from liquid retained with hydrolysed biomass upon separation of biomass and hydrolysate.

Fermentation of Hydrolysates

Each hydrolysate was fermented separately with strains K011 and SL40. Hydrolysates were prepared for fermentation immediately following clarification by adding yeast extract, tryptone, and NaCl. In addition, fermentations were conducted using LB basal medium with no added sugar, with 10% w/v xylose, and with a mixture of sugars (10% total sugars) that simulated the hydrolysates (Table 2). Both of the *E. coli* strains appeared to grow well on the hydrolysates. In fact, higher cell densities were measured for fermentations of hydrolysates than those for mixed sugars (Table 3). Maximum cell density was usually obtained approximately 24 h after inoculation.

Maximum ethanol concentrations were reached in approximately 48 h (Figures 1, 2, 3 and 4 and Table 3). They ranged in value from 3.3% w/v to 4.5% w/v and were higher for fermentations of mixed sugars compared to hydrolysates. Higher ethanol concentrations were obtained for mixed sugar fermentations because their initial total sugar concentrations were higher and/or less total sugar was left unconsumed. Virtually no ethanol was produced from fermentations of the LB basal medium without added sugar(s).

Ethanol yields varied from 0.38 g/g (g ethanol/g sugars consumed) to 0.44 g/g; between 74% and 86% of the maximum theoretical yield (Table 3). Ethanol yields were similar for hydrolysates and mixed sugar fermentations. However, strain K011 tended to give higher yields than strain SL40 for parallel fermentations. Maximum V_{EtOH} were 1.19 g/l/h to 1.99 g/l/h for both strains and were approximately a third faster for fermentation of sugar mixtures compared to hydrolysates (Table 3). The exception was fermentation of corn germ

meal hydrolysate by strain K011, for which the maximum V_{EtOH} was nearly as fast as for mixed sugars. The average maximum V_{EtOH} for all K011 fermentations was 29% faster than for that using strain SL40.

Arabinose and glucose were consumed in approximately 24 h for mixed sugars and hydrolysate fermentations; glucose was used at a faster rate than arabinose (Figures 1, 2, 3, and 4). The small amounts of galactose present in the hydrolysates were expected to be consumed nearly as quickly as glucose based upon published results (Asghari *et al.* 1996). Xylose was consumed at the slowest rate and was not completely consumed in hydrolysates or mixed sugar fermentations. *E. coli* strain K011 consumed xylose faster than strain SL40 for parallel fermentations (Figures 1, 2, 3, and 4). Concentrations of residual xylose averaged 1.2% w/v for strain SL40 and 0.5% w/v for strain K011 (Table 3). Significantly larger amounts of xylose were left unconsumed for fermentation of DietFiber® hydrolysate compared to the other fermentations (Table 3).

The amount of base (2 M KOH) added to the cultures to maintain them at pH 6.5 was 35 to 200 mmol/l culture (Table 3). Both strains produced minor amounts of organic acids, which were primarily acetate and lactate (Table 4). Only trace amounts of succinate were detected, as expected because the succinate fermentation pathway has been interrupted in these strains (Ohta *et al.* 1991). A carbon balance (Table 3) on the consumed sugars and products, including cell biomass, was used to ensure that all major fermentation products had been measured (Materials and Methods). An average of $95 \pm 3\%$ of the sugars consumed were accounted for in measured products and CO_2 .

Table 3. Fermentation data.

Substrate	<i>E. coli</i> strain	Maximum cell concentration* (g/l)	Maximum ethanol % (w/v)	Ye/s [†] (g/g)	$V_{\text{EtOH}}^{\ddagger}$ (g/l/h)	Base addition (mmole/l)	Residual sugar % (w/v)	Carbon recovery (%)
Corn fibre	K011	4.86 ± 0.01	3.47 ± 0.02	0.41 ± 0.0	1.16 ± 0.00	162.8 ± 5.7	0.57 ± 0.02	92
	SL40	4.45 ± 0.02	3.17 ± 0.03	0.42 ± 0.0	1.12 ± 0.15	120.0 ± 14.3	1.37 ± 0.13	94
Corn Germ Meal	K011	5.20 ± 0.01	3.42 ± 0.05	0.41 ± 0.01	1.88 ± 0.18	35.0 ± 20.0	0.16 ± 0.01	98
	SL40	5.30	2.92	0.38	1.19	95.0	0.70	100
Coarse Dietfiber®	K011	6.96 ± 0.42	3.76 ± 0.00	0.41 ± 0.00	1.26 ± 0.09	75.0 ± 5.0	1.58 ± 0.06	94
	SL40	6.32	3.10	0.38	1.19	75.0	2.61	92
Mixed Sugars	K011	2.43 ± 0.16	4.47 ± 0.06	0.45 ± 0.01	1.99 ± 0.04	125.0 ± 45.0	0.00 ± 0.40	100
	SL40	2.65 ± 0.14	3.26 ± 0.43	0.33 ± 0.04	1.53 ± 0.02	200.0 ± 30.0	1.39 ± 0.73	94
Xylose	K011	2.95 ± 0.16	4.42 ± 0.35	0.44 ± 0.03	1.96 ± 0.11	125.0 ± 55.0	0.00 ± 0.00	98
	SL40	2.77 ± 0.35	4.14 ± 0.45	0.42 ± 0.04	1.35 ± 0.32	134.3 ± 54.3	0.00 ± 0.00	93
LB (no sugars)	K011	0.17	0.04	NA [§]	0.04	34.29	NA	NA
	SL40	0.18	0.01	NA	0.01	34.29	NA	NA

* Cell concentration was determined by optical density at 550 nm.

[†] Yield of ethanol (g) per total consumed sugar(s) (g).

[‡] Maximum volumetric production rate of ethanol (g/l/h).

[§] NA, not applicable.

Figure 1.

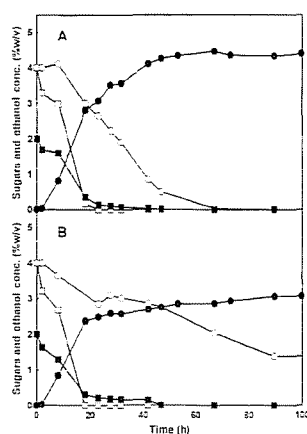


Figure 2.

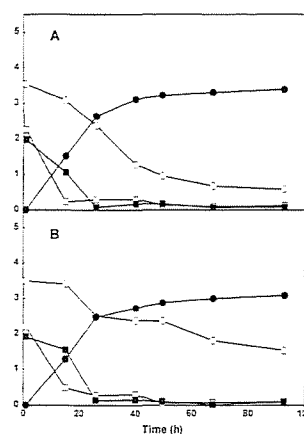


Figure 3.

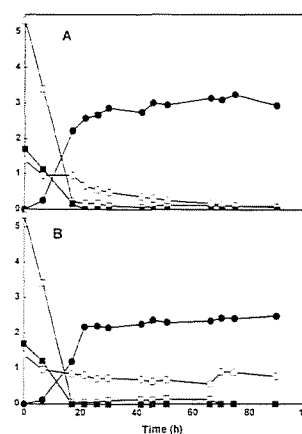


Figure 4.

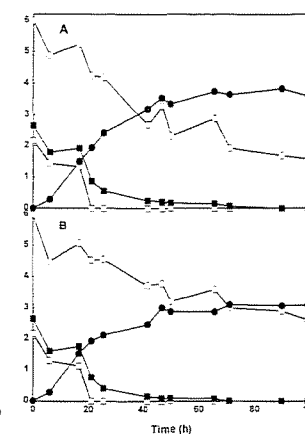


Figure 1. Fermentation of mixed sugars by *E. coli* strain K011 (A) and strain SL40 (B). **Figure 2.** Fermentation of corn fibre hydrolysate by *E. coli* strain K011 (A) and strain SL40 (B). **Figure 3.** Fermentation of corn germ meal hydrolysate by *E. coli* strain K011 (A) and strain SL40 (B). **Figure 4.** Fermentation of DietFiber® hydrolysate by *E. coli* strain K011 (A) and strain SL40 (B). ■ — Arabinose; ● — Ethanol; ○ — Xylose and Galactose; □ — Glucose.

Table 4. Organic acids present after fermentation.

Substrate	Strain	Acetate* (g/l)	Lactate (g/l)	Succinate (g/l)
Corn fibre	K011	0.5 ± 0.0	2.1 ± 0.2	0.4 ± 0.1
	SL40	0.8 ± 0.0	2.2 ± 0.0	0.6 ± 0.0
Corn germ meal	K011	2.6 ± 0.6	2.2 ± 0.0	0.6 ± 0.0
	SL40	6.4 ± 0.0	2.8 ± 0.0	0.8 ± 0.0
Coarse Dietfiber®	K011	0.35 ± 0.0	1.2 ± 0.1	1.2 ± 0.0
	SL40	1.05 ± 0.1	1.4 ± 0.3	1.1 ± 0.2
Mixed sugars	K011	2.0 ± 0.0	0.9 ± 0.1	0.8 ± 0.2
	SL40	6.0 ± 0.9	1.7 ± 0.2	1.0 ± 0.2
Xylose	K011	3.2 ± 1.5	1.1 ± 0.0	0.7 ± 0.0
	SL40	4.1 ± 3.0	0.9 ± 0.3	0.8 ± 0.1
LB (no sugars)	K011	0.7	0.0	0.7
	SL40	0.6	0.0	0.6

* Acetate concentrations detected in corn fibre and coarse DietFiber® hydrolysates before inoculation were subtracted from final measured acetate concentrations.

Discussion

The neutral non-cellulosic carbohydrate content of the corn co-products was over 50%. However, the relative amount of each sugar (arabinose, glucose, and xylose + galactose) varied widely among the biomasses. Our compositional data for corn fibre, are similar to those reported in the literature (Ghali *et al.* 1984; Koll *et al.* 1987; Osborn & Chen 1996; Wolf *et al.* 1996). In these studies, starch content was found to be 7 to 32% w/w and hemicellulose (excluding uronic acid) 29 to 41% w/w; our corn fibre was determined to contain 22% w/w starch and 47% w/w hemicellulose sugars (excluding uronic acid). The wide variation in carbohydrate contents

for corn fibre derives from the variation in starch content, which is expected to vary among mills (May 1987). Therefore, it is more meaningful to compare the ratio of xylose + galactose to arabinose, which does not vary with starch content. We measured a xylose + galactose to arabinose ratio of 1.5 g/g, which falls within the range of values previously reported of 1.4 to 2.0 g/g (Koll *et al.* 1987; Ghali *et al.* 1984; Hespell *et al.* 1996; Osborn & Chen 1996).

Weak acid proved to be an effective method for hydrolysing the feedstocks. The sugar concentrations of the hydrolysates varied from 8.4% to 10.8% w/v, which means that 60 to 77% of the possible sugars were recovered as monosaccharides. Much of the missing carbohydrates might have been lost with the pressed residual fibres, which were not washed (to avoid dilution of the hydrolysate) before being discarded. Currently, we are developing more efficient continuous weak acid hydrolysis processes.

E. coli strain K011 has been cited as a most promising fermentative microorganism for fermentation of lignocellulosic feedstocks (Hahn Hagerdal *et al.* 1994), but this conclusion was published before strain SL40 was available. Strain SL40 has been reported to have a significantly higher ethanol yield when fermenting higher initial xylose concentrations, and greater ethanol productivity than strain K011 (Lindsay *et al.* 1995). However, in parallel fermentations of the hydrolysates and mixed sugars, we have found that strain K011 consistently outperforms strain SL40. Strain K011 tended to have a higher ethanol yield, consume a greater percentage of the available sugars, and produce ethanol at a faster rate. Furthermore, when both were used to ferment a 12%

xylose solution, the final ethanol concentrations were similar (data not shown). Therefore, in our experiments, strain K011 performed better.

E. coli strain K011 fermented the corn fibre and corn germ hydrolysates efficiently and maximum ethanol concentrations were obtained within 48 h. Furthermore, ethanol yields were as good as or higher than those obtained with other microorganisms capable of fermenting mixed sugars (Hahn Hagerdal *et al.* 1993; Olsson & Hahn Hagerdal 1996). The ethanol yields for strain K011 on the hydrolysates was 0.41 g/g (g ethanol/g sugars consumed) for both corn fibre and corn germ hydrolysates, which is 80% of the theoretical maximum (0.51 g/g) and 9% lower than the yield from the mixed sugars fermentation. Fermentation of DietFiber® hydrolysate had a similar ethanol yield (0.41 g/g), but was not completed until 70 h. Previous studies have reported yields of between 0.46 g/g (Asghari *et al.* 1996) and 0.51 g/g (Beall *et al.* 1992) for the fermentation of corn hulls weak acid hydrolysate by strain K011, albeit using a different hydrolysis protocol and nitrogen source.

Maximum ethanol productivity rates for *E. coli* strains K011 and SL40 were considerably higher, often by an order of magnitude, than those reported for batch fermentations of mixed sugars using yeast or fungi (Hahn Hagerdal *et al.* 1993; Olsson & Hahn Hagerdal 1996). For hydrolysate fermentations, the maximum V_{EtOH} values were 1.16 g/l/h–1.88 g/l/h. Maximum ethanol productivity values for *E. coli* strain K011 fermenting corn germ hydrolysate and mixed sugars were similar, while corn fibre and DietFiber® hydrolysates were approximately a third slower. The corn germ hydrolysate also had the highest concentration of glucose, which these strains ferment faster than the xylose (data not shown).

E. coli strain K011 fermented arabinose and glucose rapidly and efficiently, but had difficulty fermenting xylose; xylose was consumed at the slowest rate and not completely fermented in the hydrolysates even after 100 h. The residual xylose was 2%, 6% and 15% of the initial sugars for the corn germ, corn fibre, and DietFiber® hydrolysates, respectively and was inversely proportional to initial xylose concentration. Furthermore, in the mixed sugar cultures, it took as long for 4% w/v xylose to be consumed as for 10% w/v to be consumed in the pure xylose sugar cultures. Previous studies have found that the presence of glucose in the culture inhibits xylose utilization and is associated with residual xylose (Asghari *et al.* 1996). Residual xylose is undesirable because it lowers ethanol yields and complicates disposal of the spent fermentation broth. A possible solution might be the use of a fermentation scheme similar to that used for starch fermentations. Starch fermentations are often run semi-continuously in a cascade of bioreactors (Madson & Monceaux 1995). In this way, the glucose and

arabinose could be fermented in the first tank and xylose in the subsequent tank(s).

In summary, K011 was found superior to SL40 for fermentation of both mixed sugars and hydrolysates. The best results for K011 were found in fermentations of corn germ meal and corn fibre hydrolysates, each of which contained large amounts of glucose. Coarse DietFiber® gave the least desirable results because of large amounts of residual xylose.

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